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# Ivermectin disposition kinetics after subcutaneous and intramuscular administration of an oil-based formulation to cattle

A. Lifschitz [a], G. Virkel [a], A. Pis [a], F. Imperiale [a], S. Sanchez [a], L. Alvarez [a], R. Kujanek [b], C. Lanusse [a,*]

[a] *Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro, Campus Universitario, (7000) Tandil, Argentina*

[b] *Bayer Argentina S.A, Buenos Aires, Argentina*

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**Abstract**

Slight differences in formulation may change the plasma kinetics and ecto–endoparasiticide activity of endectocide compounds. This work reports on the disposition kinetics and plasma availability of ivermectin (IVM) after subcutaneous (SC) and intramuscular (IM) administration as an oil-based formulation to cattle. Parasite-free Aberdeen Angus calves ($n=24$; 240–280 kg) were divided into three groups ($n=8$) and treated (200 μg/kg) with either an IVM oil-based pharmaceutical preparation (IVM-TEST formulation) (Bayer Argentina S.A.) given by subcutaneous (Group A) and intramuscular (Group B) injections or the IVM-CONTROL (non-aqueous formulation) (Ivomec®, MSD Agvet) subcutaneously administered (Group C). Blood samples were taken over 35 days post-treatment and the recovered plasma was extracted and analyzed by HPLC using fluorescence detection. IVM was detected in plasma between 12 h and 35 days post-administration of IVM-TEST (SC and IM injections) and IVM-CONTROL formulations. Prolonged IVM absorption half-life ($p<0.05$) and delayed peak plasma concentration ($p<0.001$) were obtained following the SC administration of the IVM-TEST compared to the IVM-CONTROL formulation. No differences in total plasma availability were observed among treatments. However, the plasma residence time and elimination half-life of IVM were significantly longer after injection of the IVM-TEST formulation. IVM plasma concentrations were above 0.5 ng/ml for 20.6 (CONTROL) and 27.5 days (IVM-TEST SC), respectively ($p<0.05$). The modified kinetic behaviour of IVM obtained after the administration of the novel oil-based formulation examined in this trial, compared to the standard

* Corresponding author. Fax: +54-2293-426667
*E-mail address:* clanusse@vet.unicen.edu.ar (C. Lanusse)

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preparation, may positively impact on its strategic use in cattle. 

*Keywords:* Ivermectin; Oil-based formulation; Pharmacokinetics; Cattle

## 1. Introduction

The avermectin family includes a series of natural and semisynthetic molecules, such as abamectin, ivermectin (IVM), doramectin and eprinomectin, which share some structural and physicochemical properties. The excellent spectrum of activity of avermectins and milbemycins against several nematode and arthropod species resulted in the all-embracing name 'endectocide', with which they are now classified (McKellar and Benchaoui, 1996). They exhibit endectocide activity at extremely low dosage rates based on a common mode of action. IVM is commercially available as injectable and pour-on formulations for use in cattle. IVM is highly effective against adults as well as developing and hypobiotic larvae of most gastrointestinal nematodes, lungworms (Egerton et al., 1981) and many arthropods in cattle (Campbell et al., 1983).

The avermectins are closely related 16-membered macrocyclic lactones, with a disaccharide substituent at $C_{13}$ (Fisher and Mrozik, 1989). IVM, a semisynthetic derivative of the avermectin family, contains a minimum of 80% 22–23 dihydroavermectin $B_{1a}$ and a maximum of 20% 22–23 dihydroavermectin $B_{1b}$. IVM is a large and highly lipophilic molecule that dissolves in most organic solvents; despite possessing two sugar rings and two hydroxyl groups, it is relatively insoluble in water (Jackson, 1989).

The pharmacokinetic behaviour of IVM has been studied in different species (Prichard et al., 1985; Fink and Porras, 1989; Bogan and McKellar, 1988; Alvinerie et al., 1993; Toutain et al., 1997). The pharmacokinetic behaviour of the drug differs according to the route of administration, formulation and animal species (Fink and Porras, 1989). The comparative plasma disposition kinetics of IVM, moxidectin and doramectin subcutaneously injected into cattle, have been characterized recently (Lanusse et al., 1997). The high lipophilicity of these molecules accounts for a wide tissue distribution and long residence in plasma, which was clearly reflected in the pharmacokinetic results obtained in those studies.

The antiparasitic spectrum and efficacy pattern of the different endectocide molecules are similar; however, differences in physico-chemical properties among them may account for differences in formulation flexibility, kinetic behaviour, and in the potency and persistence of their antiparasitic activity. It has been demonstrated that plasma availability of IVM (Lo et al., 1985) and doramectin (Wicks et al., 1993) in cattle is profoundly affected by the solvent vehicle in which the drug is formulated.

Since the antiparasite activity of endectocide molecules depends on drug concentrations and time of parasite exposure to them, an evaluation of the comparative pharmacokinetic profiles may help to estimate and optimize drug efficacy. Small differences in formulation can alter disposition kinetics, and may result in important changes in ecto–endoparasiticide activity in livestock. The goal of the study reported here was to evaluate the disposition kinetics and plasma availability of IVM following subcutaneous and intramuscular administration as a novel oil-based formulation to cattle.

## 2. Materials and methods

### 2.1. Experimental design

#### 2.1.1. Animals

The trial was conducted in 24 parasite-free Aberdeen Angus male calves, weighing 240–280 kg. All the animals were purchased from the same cattle ranch (area of Tandil, Province of Buenos Aires, Argentina). The health of the animals was monitored prior to and throughout the experimental period. Animals were in optimal nutritional condition and grazing on a lucerne/red clover pasture during the entire experimental period. They had free access to water.

#### 2.1.2. Treatments

Calves were randomly allocated into three groups of eight animals each. Treatments were given as follows:

#### 2.1.3. Group A

Experimental animals were treated with an oil-based pharmaceutical preparation of ivermectin (IVM-TEST formulation) (10 mg/ml) (Baymec Prolong®, Batch 005) provided by Bayer Argentina S.A. The treatment was given by *subcutaneous* (SC) injection in the shoulder area at 200 μg/kg body weight. This formulation contains IVM formulated in an oily vehicle and was considered as the test product in the pharmacokinetic trial.

#### 2.1.4. Group B

Exerimental animals were treated with the IVM-TEST formulation (10 mg/ml) (Baymec Prolong ®, Batch 005) provided by Bayer Argentina S.A by *intramuscular* (IM) injection in the isquio-tibial region at 200 μg/kg body weight.

#### 2.1.5. Group C

Eperimental animals were treated with a commercially available formulation of ivermectin (Ivomec®, MSD Agvet, NJ, Batch PR108) by *subcutaneous* injection in the shoulder area at 200 μg/kg body weight. This non-aqueous preparation contains IVM (10 mg/ml) formulated in a propylene glycol/glycerol formal (60 : 40) vehicle and was considered as the reference formulation (IVM-CONTROL) in the trial.

#### 2.1.6. Sampling

Blood samples were taken into heparinized vacutainer tubes prior to, and at, 0.5, 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30 and 35 days post-treatment. Blood samples were centrifuged at 2000 × *g* for 20 min and the recovered plasma was kept in labelled vials at −20°C until analyzed within 2 to 3 weeks of collection.

### 2.2. Analytical procedures

#### 2.2.1. Chemical extraction and derivatization

The extraction of IVM from spiked and experimental plasma samples was carried out following adaptations of the technique described by De Montigny et al. (1990) and Alvinerie et al. (1993); Alvinerie et al. (1995). Briefly, a 1-ml aliquot of plasma sample was combined with 100 μl of internal standard (abamectin, 100 ng/ml) and then mixed with 1-ml acetonitrile. After mixing for 20 min, the solvent-sample mixture was centrifuged at 2000 × *g* for 15 min. The supernatant was injected into a Supelclean $LC_{18}$ cartridge (Supelco, Bellfonte, PA) previously conditioned with 2 ml methanol and 2 ml deionized water. The cartridge was flushed with 2 ml of water/methanol (3 : 1). The analytes were eluted with 1 ml of methanol and concentrated to dryness under a stream of nitrogen. The reconstitution was done using 100 μl of a solution of *N*-methylimidazole (Sigma, St. Louis, MO) in acetonitrile (1 : 1). Derivatization was initiated by adding 150 μl trifluoroacetic anhydride (Sigma, St. Louis, MO) solution in acetonitrile (1 : 2). After completion of the reaction (<30 s), an aliquot (100 μl) of this solution was injected directly into the chromatograph.

#### 2.2.2. Drug analysis

IVM plasma concentrations were determined by high performance liquid chromatography (HPLC) using a Shimadzu 10 A HPLC system (Shimadzu, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase $C_{18}$ column (Phenomenex, 5 μm, 4.6 × 250 mm) kept in a column oven at 30°C (Shimadzu, Kyoto, Japan) and an acetonitrile/methanol/water (55/40/5) mobile phase at a flow rate of 1.5 ml/min. IVM was detected with a fluorescence detector (Spectrofluorometric detector RF-10, Shimadzu, Kyoto, Japan), reading at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. IVM concentrations were determined by the internal standard method using the Class LC 10 Software version 1.2 (Shimadzu, Kyoto, Japan) on an IBM compatible AT 486 computer. The IVM/abamectin peak area ratio was used to estimate the IVM concentration in spiked (validation of the analytical method) and experimental samples. There was no interference of endogenous compounds in the chromatographic determinations. The solvents (Baker, Phillipsburg, NJ) used during the extraction and drug analysis were HPLC grade.

#### 2.2.3. Validation procedures

A complete validation of the analytical procedures for extraction and quantification of IVM was performed before starting the analysis of experimental samples from the pharmacokinetic trial. IVM (Batch # 95051 2BER01, purity 97.5%) and abamectin (Batch # 9505 25BER01, purity 97.4%) reference standards provided by Bayer Argentina S.A., were used to prepare calibration curves in a range between 0.25–10 ng/ml and 10–100 ng/ml, respectively. The analytical method was validated according to the following criteria:

(a) *Linearity*: IVM and the internal standard were identified by comparison with the retention times of pure reference standards. Linearity was established to determine the concentration-detector response relationship, as determined by injection of spiked IVM standards in plasma at different concentrations (triplicate determinations). Calibration

curves were established using least-squares linear regression analysis and correlation coefficients ($r$) and coefficient of variation (CV) calculated.

(b) *Recovery*: Drug recovery was estimated from calibration lines prepared with different IVM-fortified plasma samples using abamectin as internal standard. Percentages of IVM recovery from plasma samples were obtained in the range between 0.5 and 50 ng/ml. The mean percentage of recovery and the coefficient of variation (CV) were calculated. The CV was obtained as the ratio between the standard deviations and the mean recovery values of at least three determinations.

(c) *Precision*: Inter-assay precision of the extraction and chromatography procedures was evaluated by processing replicate aliquots of pooled cattle plasma samples containing known amounts of IVM (2 and 20 ng/ml) on different days.

(d) *Detection and quantification limits*: The limit of drug detection was established with injection and HPLC analysis of plasma blanks fortified with the internal standard, measuring the baseline noise at the time of retention of the IVM peak. The mean baseline noise at the IVM retention time plus three standard deviations was defined as the detection limit. The mean baseline noise plus six standard deviations was defined as the theoretical quantification limit.

### 2.3. Pharmacokinetic and statistical analyses of the data

The plasma concentrations vs. time curves obtained after each treatment in each individual animal were fitted with PK Solutions 2.0 (Ashland, OH) computer software. Pharmacokinetic parameters were determined using a model-independent method. The peak concentration ($C_{max}$) and time to peak concentration ($T_{max}$) were read from the plotted concentration–time curve for each individual animal. The terminal (elimination) half-life ($T_{1/2el}$) and absorption half-life ($T_{1/2ab}$) were calculated as ln 2/ß and ln 2/$k_{ab}$, respectively, where ß is the terminal slope ($h^{-1}$) and $k_{ab}$ the rapid slope obtained by feathering which represents the first-order absorption rate constant ($h^{-1}$). The areas under the concentration–time curves (AUC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (ß). Statistical moment theory was applied to calculate the mean residence time (MRT) for IVM as follows

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}}$$

where AUC is as defined previously and AUMC the area under the curve of the product of time and drug concentration vs. time from zero to infinity (Gibaldi and Perrier, 1982).

IVM plasma concentrations are presented as mean ± SD. The pharmacokinetic parameters are reported as mean ± SD. Mean pharmacokinetic parameters for IVM obtained after the administration of the different formulations were statistically compared by ANOVA. Where $F$ values were significantly different, the Tukey–Kramer multiple comparisons test was applied to indicate order of significance. A value of $p < 0.05$ was considered significant.

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